

(Aus der Medizinischen Universitätsklinik Basel)

Direktor: Prof. Dr. F. KOLLER

Arbeit unter der Leitung von Dr. F. DUCKERT

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# **A Congenital Inhibitor Against Factor IX**

**INAUGURAL-DISSERTATION**

zur Erlangung der Doktorwürde der gesamten Heilkunde der  
Medizinischen Fakultät der Universität Basel

vorgelegt von

**HANS STOCKER**

von Möhlin/AG



**F. K. SCHATTAUER-VERLAG · STUTTGART 1965**



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Von der Medizinischen Fakultät genehmigt auf Antrag  
von Prof. Dr. F. KOLLER

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## CURRICULUM VITAE

Am 25. Februar 1934 wurde ich in Adliswil/ZH als drittes Kind einer Arztfamilie geboren. Nach der Absolvierung der Primarschule in Adliswil besuchte ich das Gymnasium in Zürich und Trogen/AR. Nach der Maturitätsprüfung im Herbst 1954 immatrikulierte ich mich an der Medizinischen Fakultät der Universität Zürich. 1956 legte ich das erste, 1957 das zweite propädeutische Examen ab. Im Herbst 1960 schloß ich das Studium mit dem eidgenössischen Staatsexamen an der Universität Zürich ab.

Vom 1. Oktober 1961 bis Ende November 1962 war ich am Gerinnungsphysiologischen Institut (Leitung: Prof. Dr. F. KOLLER) der Medizinischen Universitätsklinik Zürich tätig. Seit Dezember 1962 arbeite ich auf der Medizinischen Abteilung des Kreisspitals Männedorf (Chefarzt: Prof. Dr. C. MAIER).

## Introduction and Case Report

In 1958 a treatise of a hemorrhagic diathesis was published by Bütler et al. (4). The results lend them to the conclusion, that the coagulation defect from which four male members of a Swiss family suffered, was due to the deficiency of a new, unknown factor. During their studies, they asked us to do crossmatch examinations with different plasmas lacking various factors such as PTA and Hageman. Our results didn't allow us to indicate exactly the ethiology of this coagulopathy.

Nevertheless – despite the results of the other authors – we were inclined to diagnose a hemophilia B, which was indicated by the complete analysis of the blood coagulation. Additional crossmatch examinations showed, that unlike in other deficient plasmas, the coagulation- and recalcification time, as well as the consumption of the prothrombin could not be improved by addition of normal plasma in a concentration of 1:4.

This suggested that the hemorrhagic diathesis was due to an inhibitor, acting specifically against the factor IX. Hitherto all described inhibitors are acquired either by autoimmunological processes (6, 7, 13, 16, 19, 21, 23), or by the formation of antibodies after repeated transfusions (3, 22), or after pregnancy (2, 20, 28), or also through pathological increase of a normal inhibitor (9, 24).

The aim of this paper is to describe the existence of a congenital circulating inhibitor against factor IX, simulating a hemophilia B.

### *Case Report*

Since the first report (4), the family got two more boys, both affected with the same disease. Clinically it appears like a hemophilia: severe hemorrhages into joints, muscles, kidneys, subcutis etc. after slight injuries, followed by the typical secondary alterations. The first manifestations of course appeared before any transfusion.



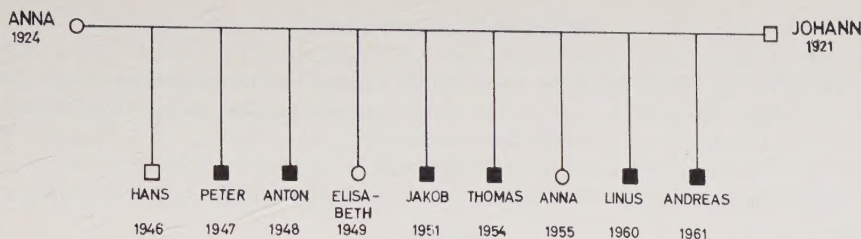


Fig. 1. Family E. with years of birth. ○ □ normal ■ bleeder.

Among the nine children, only the eldest son and the two girls didn't show any bleeding tendency. Bütler et al. state, that the father has this tendency in a very mild form. We couldn't confirm this latter finding.

A study of the family revealed a consanguinity seven generations ago (4). A blood relationship with any other known Swiss bleeders couldn't be found. The clinical symptoms and the laboratory findings were the same for all of the 6 patients. For that reason, only Anton E. (\* 1948) was subjected to a detailed clinical examination. The results were considered as representative for his brothers. The clinical examination did not reveal any pathological findings except a reduction of the movement in both foot-joints and the left elbow.

The chemical and hematological laboratory tests, especially the examination of the liver functions, including Vitamin K - test (14), and the determination of various enzymes such as transaminase, alkaline phosphatase and lactat-dehydrogenase, were normal. The complete analysis of the blood coagulation again pointed to a hemophilia B (Tab. 1).

Table 1. Clotting Tests of Anton E.

|                                                                |         | Normal        |
|----------------------------------------------------------------|---------|---------------|
| Coagulation time (Lee-White) . . . . .                         | > 60'   | 7 - 10'       |
| Bleeding time (Duke) . . . . .                                 | 2' 30'' | 2 - 5'        |
| Rumpel-Leede . . . . .                                         | neg.    |               |
| Quick . . . . .                                                | 46%     | 70 - 100%     |
| Recalcification time . . . . .                                 | 430''   | 60 - 120''    |
| Residual prothrombin in Serum . . . . .                        | 100%    | 1 - 7%        |
| Fibrinogen . . . . .                                           | 350 mg% | 250 - 500 mg% |
| Prothrombin . . . . .                                          | 100%    | 70 - 100%     |
| Factor V . . . . .                                             | 100%    | 50 - 100%     |
| Factor VII . . . . .                                           | 36%     | 70 - 100%     |
| Factor VII - complex . . . . .                                 | 50%     | 70 - 100%     |
| Factor VIII . . . . .                                          | 100%    | 50 - 100%     |
| Factor IX . . . . .                                            | ≤ 1%    | 50 - 100%     |
| Factor X . . . . .                                             | 100%    | 70 - 100%     |
| Thromboplastin generation test:                                |         |               |
| BaSO <sub>4</sub> normal plasma + normal serum . . . . .       | 11''    | 11''          |
| BaSO <sub>4</sub> patient's plasma + normal serum . . . . .    | 11''    |               |
| BaSO <sub>4</sub> normal plasma + patient's serum . . . . .    | 48''    |               |
| BaSO <sub>4</sub> patient's plasma + patient's serum . . . . . | 54''    |               |
| Antithrombin . . . . .                                         | 17''    | 13 - 18''     |

## Materials and Methods

1. The *laboratory assay methods* are used for the quantitative determination of the clotting factors. The values are given in units, with normal oxalated plasma taken as a standard with 100 u/ml. The fibrinogen concentration is reported in mg %.

- Fibrinogen determination according to Clauss (5).
- Prothrombin and Factor VII complex determinations according to Koller, Loeliger and Duckert (15).
- Factor VII determination according to Zollinger (29).
- Factor VIII and IX determination according to Geiger, Duckert and Koller (12).
- Factor X determination according to Bachmann, Duckert and Koller (1).
- Veronal - acetate buffer pH 7.35 according to Michaelis (1).

2. *Cross-match examinations.* The blood was collected by venipuncture. To 1.7 ml whole blood we added 0.2 ml plasma or serum. To neutralize the sodium-oxalate in the plasma, we added 0.1 ml  $\text{CaCl}_2$  m/40. When using serum, we added 0.1 ml veronal-acetate buffer pH 7.35 instead of  $\text{CaCl}_2$ . After incubation for one hour at  $37^\circ \text{C}$ , the blood was left at room-temperature for a total of 24 hours, then centrifuged and the serum stored at  $-25^\circ \text{C}$  in glass tubes. After thawing we determined the prothrombin and the one-stage factor IX.

The average hematocrit of the patients being 48, the share of the plasma in the mixture is about 0.8 ml. Consequently the proportion of patient plasma to added plasma resp. serum is 4:1.

3. *Intermediate product I generation test.* In the present experiment, the basic system consists in the  $\text{BaSO}_4$  adsorbed plasma and normal serum diluted  $\frac{1}{3}$  (as factor X donor), or purified factor X, when clot-promoting-euglobulin fractions are tested. In some experiments the undiluted normal serum of the original incubation system is replaced by variable amounts of the tested serum.

Original incubation system:

- 0.2 ml  $\text{BaSO}_4$  adsorbed plasma (undiluted)
- 0.2 ml serum (undiluted)
- 0.5 ml buffer
- 0.1 ml  $\text{CaCl}_2$  m/20

The intermediate product I - activity generated in the incubation mixture is determined by recording the clotting time after adding - at fixed intervals - 0.1 ml of the incubation mixture to aliquots of Ca-free plasma (8). Activity of intermediate product I in per cent is referred to a normal system.

4. *Rapid generation test according to Matter* (18). The test measures the thrombin- and the intrinsic activator activity evolved in diluted whole plasma.

To obtain platelet factor 3, the platelets of sedimented oxalated plasma have to be destroyed mechanically with a glass rod. Then the platelet factor 3 rich plasma is diluted with buffer in the proportion of 1:9 and recalcificated with  $\text{CaCl}_2$  m/40. The mixture is incubated at  $37^\circ \text{C}$ . The thrombin activity generated in the incubation mixture is determined by the same principle as the intermediate product I activity.

In a modification we replaced the platelet rich plasma by centrifuged plasma diluted with buffer containing phospholipids (chloroform brain extract).

5. *Modified factor IX determination according to Geiger* (11).

- 0.2 ml oxalated plasma
- 1.4 ml buffer
- 0.2 ml thrombokinasase diluted 1:10 in buffer
- 0.2 ml  $\text{CaCl}_2$  m/40.

After coagulation at room temperature, the mixture is incubated for 4 hours at  $37^\circ \text{C}$ . After-

wards the serum is centrifuged at 25,000 g during 1 hour, and the factor IX activity is determined without further dilution by the one stage assay according to Geiger, Duckert and Koller (12).

6. *Thromboplastin generation test* in a modification according to Duckert, Flückiger, Isenschmid, Matter, Vogel-Meng and Koller (8).

7. *Paper electrophoresis* according to Fisch (10).

8. *Starch gel electrophoresis* according to Straub (25).

## Results

As mentioned in the introduction, the coagulation- and recalcification time as well as the prothrombin consumption of patient's plasma were not improved by addition of normal plasma. Therefore we performed cross-match examinations with different normal – and deficient plasmas and sera, measuring the coagulation time (Lee-White), the residual serum prothrombin and the one stage factor IX activity in the serum.

Normal plasma added to the patient's plasma is unable to enhance the blood coagulation (Tab. 2). The formation of intrinsic activator is not or insignificantly improved, which is shown as well in the prolonged coagulation time as

Table 2. Cross-Match Examinations.

| Admixture                            | Clotting-time<br>(Lee-White) | Residual pro-<br>thrombin in<br>serum<br>(normal 1–7%) | Factor IX or its precursor |          |
|--------------------------------------|------------------------------|--------------------------------------------------------|----------------------------|----------|
|                                      |                              |                                                        | calculated                 | measured |
| Patient's plasma without admixture   | > 60'                        | > 100%                                                 |                            | ≤ 1%     |
| Normal plasma                        | > 60'                        | 72%                                                    | 20%                        | 3%       |
| Normal plasma 1/2 diluted            | > 60'                        | 78%                                                    | 10%                        | 2%       |
| Hemophilia A plasma I                | < 10'                        | 1%                                                     | 20%                        | 22%      |
| Hemophilia A plasma II               | < 10'                        | 1%                                                     | 20%                        | 25%      |
| Hemophilia A plasma III              | < 10'                        | 1%                                                     | 20%                        | 25%      |
| Hemophilia A plasma IV               | < 10'                        | 1%                                                     | 20%                        | 20%      |
| Hemophilia A plasma V                | < 10'                        | 1%                                                     | 20%                        | 22%      |
| Hemophilia A plasma VI               | < 10'                        | 1%                                                     | 20%                        | 19%      |
| Hemophilia B serum                   | > 60'                        | 88%                                                    | ≤ 1%                       | 1 1/2%   |
| Normal serum I                       | < 10'                        | 1%                                                     | 20%                        | 30%      |
| Normal serum II                      | < 10'                        | 1%                                                     | 20%                        | 46%      |
| Normal serum III                     | < 10'                        | 1%                                                     | 20%                        | 35%      |
| Normal serum IV <sup>1)</sup>        | < 10'                        | 1%                                                     | 20%                        | 50%      |
| Buffer                               | > 60'                        | 78%                                                    | ≤ 1%                       | ≤ 1%     |
| Glass-wool as activator              | < 10'                        | 3%                                                     | ≤ 1%                       | ≤ 1%     |
| Patient's plasma +<br>+ thrombokinas | < 10'                        | 1%                                                     | ≤ 1%                       | ≤ 1%     |

1) This serum was used for the calibration curve for the factor IX determination.

in the low prothrombin consumption. Likewise the obtained factor IX activity is only 3% instead of the calculated 20%. On the other hand we found a normal coagulation time and complete prothrombin consumption after addition of different hemophilia A – plasmas and normal sera (in both the factor VIII activity is  $<1\%$ ). Here the Factor IX activity corresponds to the calculated one. Therefore we supposed, that the plasma of the patients has likely a normal factor IX activity beside an unknown inhibitor.

If hemophilia B – blood (factor IX  $<1\%$ ) is taken instead of patient's blood, the addition of normal – or hemophilia A – plasma or normal serum makes no difference: in any case we got a normal coagulation time, a complete prothrombin consumption and a factor IX activity of 20% as calculated.

To prove the presence of factor IX in a sufficient concentration in the patient's plasma, we isolated the clot-promoting euglobulin fraction (CPE) from normal-hemophilia B- and patient's serum by the method of Yin and Duckert (26). Besides the factor IX or its precursor CPE contains also the factor XI (PTA) and XII (Hageman). The fractions were tested in the intermediate product I formation test. As a factor X source, we used normal serum diluted  $\frac{1}{3}$  (Fig. 2) and purified factor X (Fig. 3).

The illustrations show, that the CPE-fractions from patient's – and normal serum act identically: after a normal lag period – due to the activation and reaction of Hageman factor with PTA factor- a normal intermediate product I formation takes place. Because of the low IX concentration, serum diluted  $\frac{1}{3}$  or purified factor X are unable to form intermediate product I. The CPE-fraction

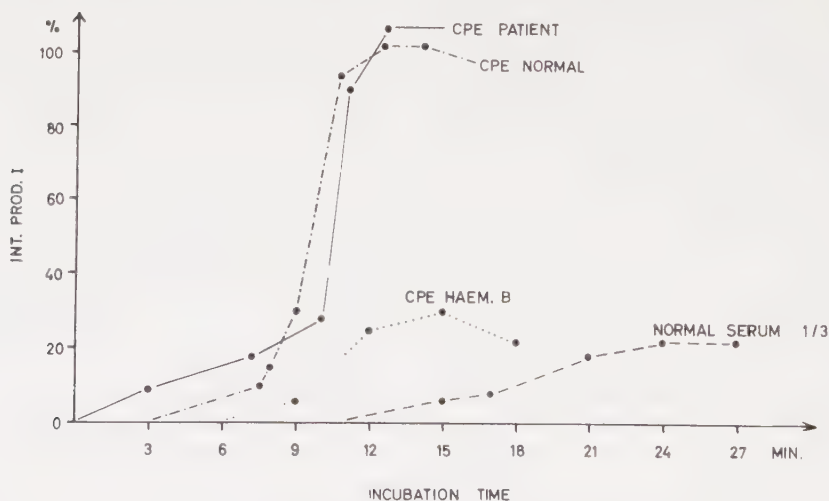


Fig. 2. Intermediate product I generation. Effect of clot promoting euglobulin fraction (CPE) on serum diluted  $\frac{1}{3}$  (see text).



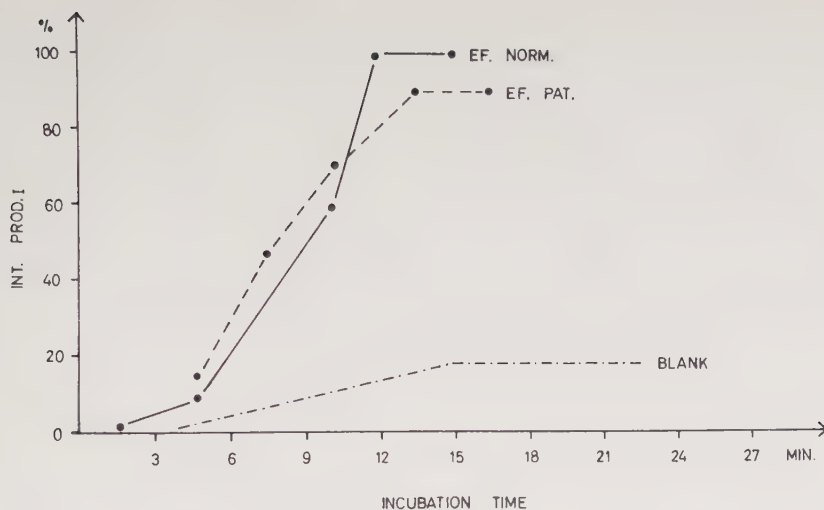


Fig. 3. Intermediate product I generation. Effect of CPE on purified factor X.

of hemophilia B serum, devoid of factor IX, cannot produce intermediate product I. These results and those of the cross-matchings give the evidence, that factor IX, in a hidden but physiological active form, is present in the patient's plasma. Consequently a hemophilia B is excluded.

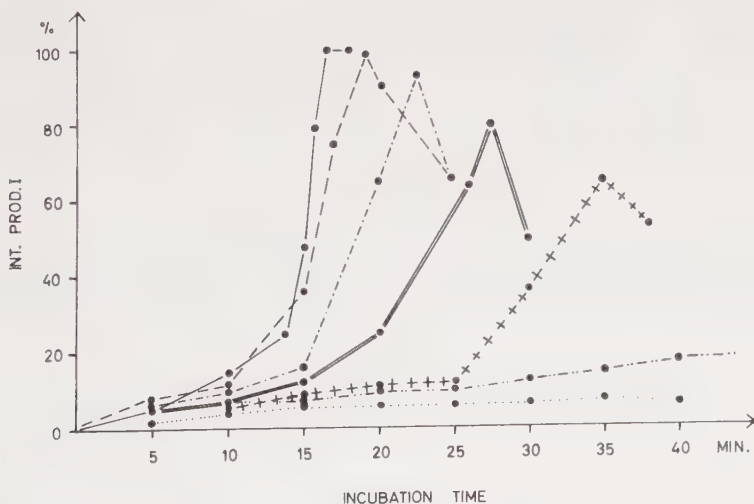


Fig. 4. Effect of variable concentrations of patient's serum on the intermediate product I generation. ——— 0.2 ml normal serum; ——— 0.18 ml normal serum + 0.02 ml patient's serum; - - - - - 0.15 ml normal serum + 0.05 ml patient's serum; = = = = 0.1 ml normal serum + 0.1 ml patient's serum; + + + + 0.05 ml normal serum + 0.15 ml patient's serum; - - - - - 0.02 ml normal serum + 0.18 ml patient's serum; ····· 0.2 ml patient's serum.

If inhibitor is present in the patient's plasma and -serum, its addition to a normal system indicate a significant inhibition of the thromboplastin formation. We therefore tested in the intermediate product I formation test the patient's serum which was added in variable concentrations to normal serum. The effect on the yield of the intermediate product I and on the lag period was measured.

As can be seen in Fig. 4 the lag period is prolonged and the yield of intermediate product I is reduced by the influence of the inhibitor in the prephase. As shown by Yin and Duckert (27), a dilution of factor IX has no influence on the lag period, but only on the yield of the intermediate product I formation. In each mixture a 100% yield of intermediate product I was expected, because, as demonstrated, factor IX is present in normal amount in the patient's serum.

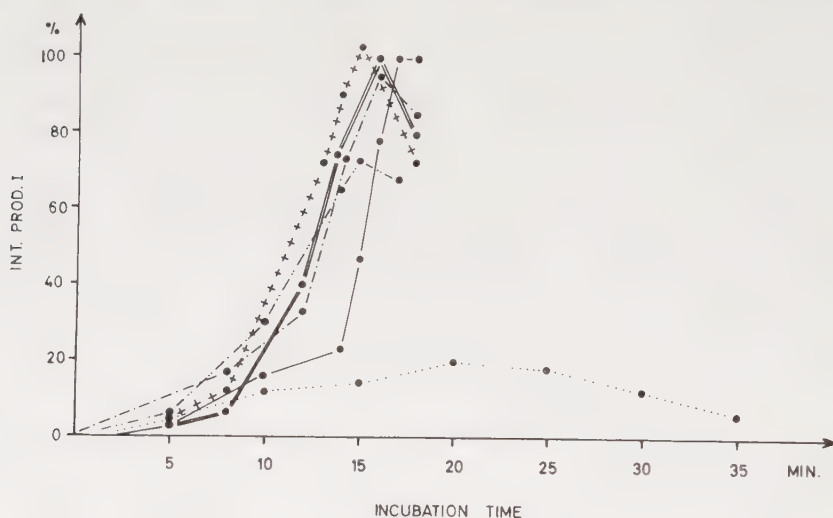


Fig. 5. Effect of variable concentration of hemophilia B serum on the intermediate product I generation. ——— 0.2 ml normal serum; - - - - - 0.15 ml normal serum + 0.05 ml hemophilia B-serum; = = = 0.1 ml normal serum + 0.1 ml hemophilia B-serum; + + + + 0.05 ml normal serum + 0.15 ml hemophilia B-serum; ······ 0.02 ml normal serum + 0.18 ml hemophilia B-serum; ..... 0.2 ml hemophilia B-serum.

With hemophilia B serum instead of patient's serum (Fig. 5), strongly diluted normal serum is able to produce a nearly complete yield of intermediate product I after a normal lag period. This yield is much higher as expected. The cause is a high concentration of prothrombin in hemophilia B serum, which allows a certain formation of thrombin in the incubation mixture. On the other hand, the concentration of prothrombin in the patient's serum, equivalent to that of hemophilia B serum, was strong enough to simulate a pseudo-normalization by formation of thrombin.

In order to eliminate disturbing elements, the further investigations were performed exclusively with plasma, which was given in variable proportions to patient's plasma or hemophilia B plasma. Such conditions are found in the rapid generation test of Matter (18), where thrombin activity is produced and measured.

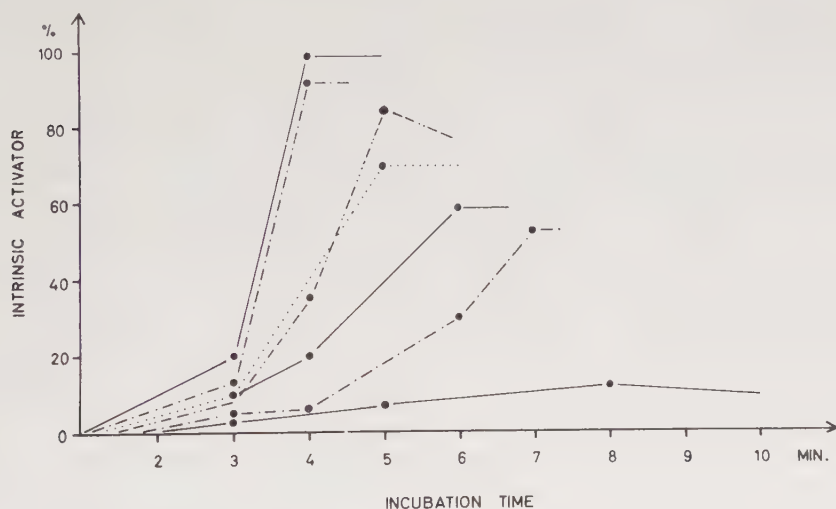


Fig. 6. Rapid generation test according to Matter. Effect of variable concentrations of patient's plasma on the thromboplastin formation. — 0.2 ml normal plasma; - - - - 0.18 ml normal plasma + 0.02 ml patient's plasma; - · - · - 0.15 ml normal plasma + 0.05 ml patient's plasma; · · · · · 0.1 ml normal plasma + 0.1 ml patient's plasma; ——— 0.05 ml normal plasma + 0.15 ml patient's plasma; - - - - 0.02 ml normal plasma + 0.18 ml patient's plasma; ——— 0.2 ml patient's plasma.

Patient's plasma is able to inhibit the thrombin formation even at low concentrations (Fig. 6). On the contrary, normal plasma in the same concentration guarantees a nearly normal in vitro-coagulation when added to hemophilia B plasma (Fig. 7).

In the following experiments, patient's plasma is mixed with normal plasma respectively hemophilia A plasma. By the method of Geiger (11) we afterwards obtained the recalcification-serum, in which we measured the factor IX activity (Tab. 3).

The factor IX activities obtained in the mixtures with normal plasma are below the calculated levels, whereas by addition of hemophilia A plasma the factor IX activity is higher. This observation was already made in the cross-match examinations.

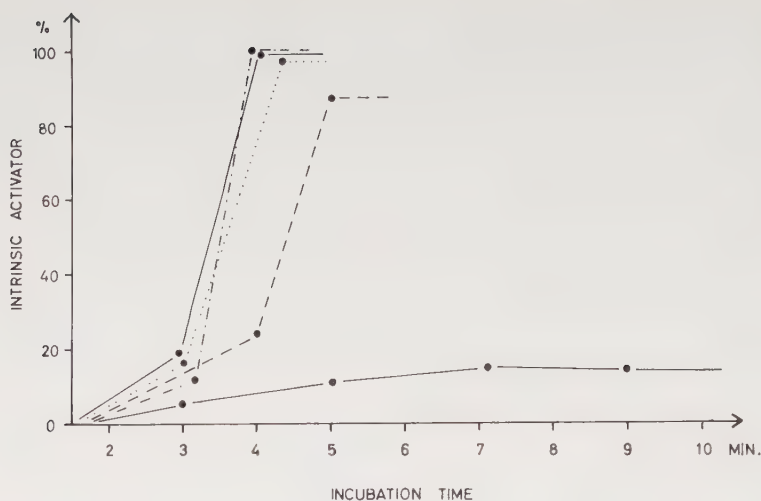


Fig. 7. Rapid generation test according to Matter. Effect of variable concentrations of hemophilia B plasma on the thromboplastin formation. — 0.2 ml normal plasma; - - - - 0.1 ml normal plasma + 0.1 ml hemophilia B plasma; ····· 0.05 ml normal plasma + 0.15 ml hemophilia B plasma; - · - · - 0.02 ml normal plasma + 0.18 ml hemophilia B plasma — 0.2 ml hemophilia B plasma.

Table 3. Factor IX Determination in Recalcification Serum. The Serum is Obtained from Various Mixtures of Patient's Plasma with Normal Plasma or Hemophilia A-Plasma.

| Proportion of       |                  | Factor IX activity |          |
|---------------------|------------------|--------------------|----------|
| normal plasma       | patient's plasma | calculated         | measured |
| 1                   | 9                | 10.0%              | 6%       |
| 1                   | 3                | 25.0%              | 16%      |
| 1                   | 1                | 50.0%              | 40%      |
| 3                   | 1                | 75.0%              | 65%      |
| 10                  | —                | 100.0%             | 100%     |
| hemophilia A-plasma | patient's plasma | calculated         | measured |
| 1                   | 9                | 11.0%              | 15%      |
| 1                   | 3                | 27.5%              | 40%      |
| 1                   | 1                | 55.0%              | 80%      |
| 3                   | 1                | 82.5%              | 100%     |
| 10                  | —                | 110.0%             | 110%     |

These findings suggest, that the inhibitor is in excess. Therefore it is able to inactivate besides the proper factor IX an important part of the factor IX supplied with the normal plasma. On the other hand, we made the striking observation, that the effect of the inhibitor is much less in a factor VIII-poor system (cf. Tab. 2, 3).



## Isolation and Physico-Chemical Properties of the Inhibitor

1. *Isolation* by means of paper electrophoresis and starch gel electrophoresis.

The trials to isolate the inhibitor by means of the paper electrophoresis (10) and the starch gel electrophoresis (25) failed. In none of the tested fractions the inhibitor was demonstrable. Probably the failure is caused by the lability of the inhibitor, which first loses its specific activity during the 12 resp. 21 hours lasting electrophoresis at a temperature of 20–25° C and at high pH, and then during the elution out of the paper or starch. The final concentration wasn't any more sufficient for a significant inhibition of the test system.

2. *Influence of temperature.* Heated for 30' at 56° C the inhibitor is destroyed, whereas it is stable during one year when stored at –20° C.

3. *Adsorption and elution.* The inhibitor can be adsorbed completely on  $\text{Al}(\text{OH})_3$  and  $\text{BaSO}_4$  (Fig. 8 and 9).

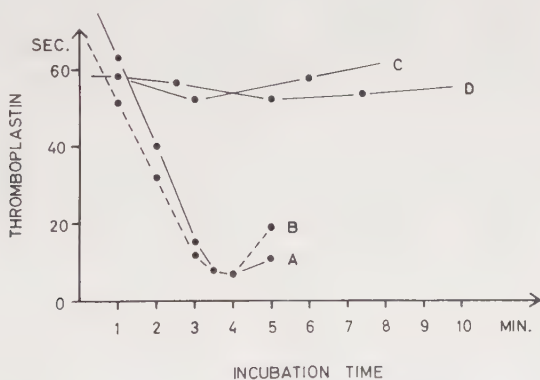


Fig. 8. Complete adsorption of the inhibitor on  $\text{BaSO}_4$  shown in the thromboplastin generation test. A.  $\text{BaSO}_4$ -adsorbed normal plasma + normal serum; B.  $\text{BaSO}_4$ -adsorbed patient's plasma + normal serum; C.  $\text{BaSO}_4$ -adsorbed normal plasma + patient's serum; D.  $\text{BaSO}_4$ -adsorbed patient's plasma + patient's serum.

The inhibitor adsorbed on  $\text{BaSO}_4$  can be eluted with 0.14 M sodium-citrate without losing its specific character. For that purpose we treated oxalated normal- and patient's serum with  $\text{BaSO}_4$ . In the supernatant remains -besides an important part of the factor IX- considerable quantities of the factor XI and XII. On the other hand, the inhibitor like the factors VII, X, some IX and the PPA adsorbed on  $\text{BaSO}_4$ , can be eluted with sodium-citrate. By adding equal parts of supernatant after  $\text{BaSO}_4$  adsorption and eluate of the  $\text{BaSO}_4$  a complete serum is obtained, which was tested afterwards in the thromboplastin generation test (TGT) (8).

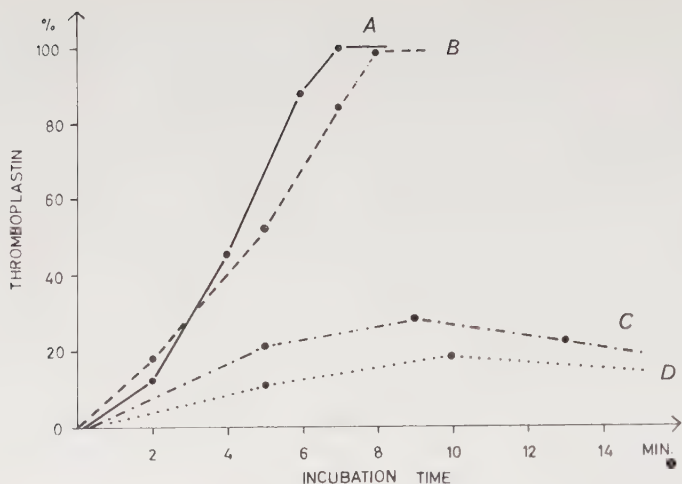


Fig. 9. Effect of eluate of  $\text{BaSO}_4$ -treated patient's serum on the thromboplastin generation test. — normal plasma +  $\text{BaSO}_4$ -treated normal serum + eluate of normal serum; ----- normal plasma +  $\text{BaSO}_4$ -treated patient's serum + eluate of normal serum; - · - · - normal plasma +  $\text{BaSO}_4$ -treated normal serum + eluate of patient's serum; · · · · · normal plasma +  $\text{BaSO}_4$ -treated patient's serum + eluate of patient's serum.

Fig. 9 shows in per cents, the amount of thromboplastin obtained in the TGT with different mixtures of serum. Curve A corresponds to normal serum. Curve B indicates, that a mixture of  $\text{BaSO}_4$ -adsorbed patient's serum and eluate of normal serum gives a complete serum. This is only possible, when the patient's serum contains efficient factor IX, and when the inhibitor is completely adsorbed on  $\text{BaSO}_4$ .

As expected, the eluate of the patient's serum inhibits the physiological factor IX activity of normal serum treated with  $\text{BaSO}_4$  (Curve C).

4. *Precipitation with  $(\text{NH}_4)_2\text{SO}_4$ .* After precipitation at variable degrees of saturation, the presence of inhibitor can't be demonstrated in any of the fractions. One can assume, that it is denaturated by  $(\text{NH}_4)_2\text{SO}_4$ .

## Discussion

The serious congenital hemorrhagic diathesis from which 6 boys of a Swiss family are suffering, seemed - according to the usual analysis of the clotting system - to be caused by a deficiency of factor IX. Usually the coagulation- and recalcification time of a deficient plasma are improved by addition of normal plasma at a final concentration of 20%. In this respect, the plasma of our patients differs from a hemophilia B, so we presumed an inhibitor with a selective inhibition of the factor IX.

A hemophilia B can be excluded because of the presence of factor IX in the patient's serum (Fig. 2, 3 and 9 Curve B). Although its isolation failed, the presence of the inhibitor is proved by the evident inhibition of a normal clotting system after addition of patient's plasma resp. -serum (Fig. 4, 6 and 9, Curve C, Tab. 3).

On the other hand an activation of the factor IX takes place, when the patient's blood is mixed with hemophilia A plasma respectively normal serum, whereas the addition of normal plasma of deficient plasma containing factor VIII shows only a very slight correcting effect (Tab. 2 and 3). The same observation – normalization of the TGT and the recalcification time by addition of hemophilia A plasma and normal serum to patient's blood – has already been made by Bütler et al. They supposed, that the presumably deficient factor was substituted.

It appears, that the activity of the inhibitor depends on the factor VIII concentration. At a low factor VIII level, the inhibitor cannot develop its full activity. In 1959 an acquired antithromboplastin in a case of Lupus erythematosus disseminatus was described by Loeliger (17). This antithromboplastin – in analogy to our inhibitor – requires the presence of prothrombin in order to attain its full activity.

To enlighten the phenomen, we made the following hypothesis: If the inhibitor is associated to the factor VIII, it is able to develop its specific action, whereas, if it is dissociated, it loses this ability. An equilibrium must exist between the effective inhibitor, that means inhibitor associated to factor VIII, and the dissociated-one:

$$\frac{(\text{factor VIII}) \times (\text{inhibitor})}{(\text{factor VIII} \times \text{inhibitor})} = k$$

Consequently, in a system poor of factor VIII, the effective inhibitor (inhibitor  $\times$  factor VIII) is diminished.

Therefore the cross-match examinations and the results of the recalcification-serum can be explained as follows:

|                                             |                        |                                                                                     |                                 | IX-activity |
|---------------------------------------------|------------------------|-------------------------------------------------------------------------------------|---------------------------------|-------------|
| Patient's plasma                            | VIII-Inhibitor complex | +                                                                                   | dissociated inhibitor in excess | $\leq 1\%$  |
| Patient's plasma +<br>+ normal plasma       | VIII-Inhibitor complex |  | dissociated inhibitor           | 3%          |
| Patient's plasma +<br>+ hemophilia A-plasma | VIII-Inhibitor complex |  | dissociated inhibitor           | 20–25%      |
| Patient's plasma +<br>+ normal serum        | VIII-Inhibitor complex |  | dissociated inhibitor           | 30–50%      |
| Patient's plasma +<br>+ hemophilia B-serum  | VIII-Inhibitor complex |  | dissociated inhibitor           | 1.5%        |

In pure patient's blood the factor IX activity is  $\leq 1\%$ , because it is repressed completely by the active inhibitor (factor VIII  $\times$  inhibitor). Furthermore an excess of dissociated inhibitor is present. By addition of normal plasma to patient's blood, the surplus of dissociated inhibitor combines with the factor VIII of the normal plasma. The additionally formed inhibitor impairs consequently the factor IX of the normal plasma. On the other hand, the equilibrium is displaced in favour of ineffective dissociated inhibitor, when hemophilia A plasma or normal serum are mixed to the patient's blood.

Effective inhibitor is present in a sufficient concentration, to hinder the factor IX activation in the patient's blood. But there is not enough effective inhibitor to suppress the activation of the factor IX supplied with the hemophilia A plasma resp. normal serum.

The presence of activated products in the serum is responsible for the higher rate of activated IX when serum is added. For this reason a slight activation of factor IX takes place by addition of hemophilia B serum. The inhibitor is still strong enough to inhibit the patient's own factor IX.

The factor VIII determination (12) being performed in a system poor of factor VIII, the hypothetical complex factor VIII  $\times$  inhibitor is therefore dissociated and the factor VIII can be measured. On that account we get a factor VIII activity of 90–100%, even when a certain part will be denaturated or inactivated. For this reason it is impossible to say whether factor VIII in the complex is active or not.

The exact nature of the relation between factor VIII and the inhibitor is not definitively elucidated. Nevertheless we were able to demonstrate the existence of a congenital inhibitor as ethiological agent of this serious hemorrhagic diathesis.

### Summary

A new congenital hemorrhagic diathesis, which is due to the presence of a circulating inhibitor against factor IX, simulating a hemophilia B, is described. It is demonstrated, that the inhibitor becomes much less efficient when the factor VIII-concentration decreases. A hypothesis is given which should explain the role of factor VIII in the inhibition process.

### Résumé

Une nouvelle diathèse hémorragique congénitale, due à la présence d'un inhibiteur du facteur IX, simulant une hémophilie B, est décrite. Il est démontré, que l'activité de l'inhibiteur diminue quand la concentration du facteur VIII diminue. On tente au moyen d'une hypothèse, d'expliquer le rôle du facteur VIII dans ce processus d'inhibition.



## Zusammenfassung

Beschreibung eines zirkulierenden, gegen Faktor IX gerichteten Hemmkörpers. Gerinnungsphysiologisch und klinisch wird eine schwere Hämophilie B vorgetäuscht. Es konnte gezeigt werden, daß der Hemmkörper bedeutend weniger aktiv wird, wenn die Faktor-VIII-Konzentration abnimmt. Eine Hypothese versucht, die Rolle des Faktors VIII zu klären.

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